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**Differentiation of V2a interneurons from human pluripotent stem cells.**

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**Public Summary:**

In our recent PNAS publication entitled "Differentiation of V2a interneurons from Pluripotent Stem Cells", we described the first protocol to generate V2a interneurons from human pluripotent stem cells, a cell population critical to the coordination of movement and breathing. We demonstrate that this population matures in vitro and extends long neurites when transplanted into uninjured murine spinal cords.

**Scientific Abstract:**

The spinal cord consists of multiple neuronal cell types that are critical to motor control and arise from distinct progenitor domains in the developing neural tube. Excitatory V2a interneurons in particular are an integral component of central pattern generators that control respiration and locomotion; however, the lack of a robust source of human V2a interneurons limits the ability to molecularly profile these cells and examine their therapeutic potential to treat spinal cord injury (SCI). Here, we report the directed differentiation of CHX10<sup>+</sup> V2a interneurons from human pluripotent stem cells (hPSCs). Signaling pathways (retinoic acid, sonic hedgehog, and Notch) that pattern the neural tube were sequentially perturbed to identify an optimized combination of small molecules that yielded approximately 25% CHX10<sup>+</sup> cells in four hPSC lines. Differentiated cultures expressed much higher levels of V2a phenotypic markers (CHX10 and SOX14) than other neural lineage markers. Over time, CHX10<sup>+</sup> cells expressed neuronal markers [neurofilament, NeuN, and vesicular glutamate transporter 2 (VGlut2)], and cultures exhibited increased action potential frequency. Single-cell RNAseq analysis confirmed CHX10<sup>+</sup> cells within the differentiated population, which consisted primarily of neurons with some glial and neural progenitor cells. At 2 wk after transplantation into the spinal cord of mice, hPSC-derived V2a cultures survived at the site of injection, coexpressed NeuN and VGlut2, extended neurites >5 mm, and formed putative synapses with host neurons. These results provide a description of V2a interneurons differentiated from hPSCs that may be used to model central nervous system development and serve as a potential cell therapy for SCI.

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